American Fern Journal 99(4):292–306 (2009)

Mycorrhizal Associations in Ferns from Southern Ecuador

MARCUS LEHNERT*
Albrecht-von-Haller-Institut für Pflanzenwissenschaften, Abt. Systematische Botanik, Georg-August-Universität Göttingen, Untere Karspüle 2, D-37073 Göttingen, Germany
INGRID KOTTKE and SABRINA SETARO
Botanisches Institut, Spezielle Botanik, Mykologie und Botanischer Garten, Eberhard-Karls-Universität, Auf der Morgenstelle 1, D-72076 Tübingen, Germany
LINDA F. PAZMIÑO and JUAN PABLO SUÁREZ
Escuela de Ciencias Ambientales, Universidad Técnica Particular de Loja, Ecuador
MICHAEL KESSLER[1]
Albrecht-von-Haller-Institut für Pflanzenwissenschaften, Abt. Systematische Botanik, Georg-August-Universität Göttingen, Untere Karspüle 2, D-37073 Göttingen, Germany

ABSTRACT.—We conducted a survey on the mycorrhizal status of neotropical ferns, focusing on previously neglected taxa. These include the filmy ferns (Hymenophyllaceae), grammitid ferns (Polypodiaceae), and the genus *Elaphoglossum* (Dryopteridaceae). Samples were collected at different sites in southern Ecuador, Prov. Loja, Morona-Santiago, and Zamora-Chinchipe. Among the 85 investigated species (101 samples, 10 families), 19 were associated with arbuscular mycorrhizal fungi (AMF) and 36 were infected by dark septate endophytes (DSE), which are identified as ascomycetes and here considered as a kind of mycorrhiza similar to the ericoid type. The roots of 30 species (including all non-grammitid Polypodiaceae and half of the *Elaphoglossum* species) were free of evident fungal infection. AMF were frequent in terrestrial species (29.10% of species, or 48.49% of infected terrestrial samples). DSE prevailed in epiphytic species (58.62% of species, or 96.15% of infected epiphytic samples) and were also common in terrestrial samples of predominantly epiphytic species.

KEY WORDS.—Andes, arbuscular mycorrhizal fungi (AMF), ascomycetes, dark septate endophytes (DSE), grammitid ferns, Hymenophyllaceae, vesicular arbuscular mycorrhizae (VAM)

Mycorrhiza, the symbiosis between fungi and plant root, is known to enable plants to survive in the harshest environments by mediating nutrient and water fluxes (Allen *et al.*, 2003; Cairney and Meharg, 2003; Cooke and Lefor, 1998). Despite the evident advantage, there are conditions under which plants may dispense of a fungal partner and thrive, especially if they are growing on substrates with easy nutrient availability. Since most plant groups have a preference for one type of substrate, it does not surprise that mycorrhizae are

*Corresponding author new address: Staatliches Museum für Naturkunde Stuttgart, Am Löwentor, Rosenstein 1, D-70191 Stuttgart, Germany; email: lehnert.smns@naturkundemuseum-bw.de
[1]New address: Systematic Botany, University of Zürich, Zollikerstrasse 107, CH-8008 Zürich, Switzerland.

unevenly distributed among the plant families (Newman and Reddell, 1987; Wang and Qui, 2006). Each new screening for fungal infections helps to understand the relationship between substrate type and mycorrhizae, especially if they include exceptions from the rule (e.g., Gemma *et al.*, 1992; Moteetee *et al.*, 1996).

Mycorrhization is common and diverse among landplants (Brundrett, 2002, 2004; Allen *et al.*, 2003) but only two types have been confirmed for ferns and lycophytes. The arbuscular mycorrhizal fungi (AMF) belong exclusively to the Glomeromycota (Schüßler *et al.*, 2001; Brundrett, 2004) and are the oldest form of the symbiosis (Pirozynski and Malloch, 1975; Blackwell, 2000; Brundrett, 2002). They are prevailing among ferns, lycophytes, and most other groups of vascular land plants (Brundrett, 2004). AMF are unable to grow without the association to a green plant (Brundrett, 2002), and are not easily dispersed from the soil to other habitats (Janos, 1993). The other group is the dark septate endophytes (DSE), which is a polyphyletic compound of several more derived fungal lineages. Contrary to the AMF, their spores get airborne more easily and are thus more readily available in the epiphytic habitat. The symbiotic character of DSE associations is still discussed controversially because the taxa involved are closely related to non-symbiotic endophytes, pathogens, and litter decomposers (Jumpponen and Trappe, 1998). However, most DSE found in ferns are apparently related to the ascomycetes (Schmid *et al.*, 1995) that form the well-studied Ericoid mycorrrhiza (Cairney and Meharg, 2003). Basidiomycetes (i.e., the known showy mushrooms) are commonly associated with northern temperate tree species and most orchids, including the epiphytic species (Brundrett 2004). Although they can also be found in liverworts (Kottke and Nebel, 2005), they are not confirmed as fungal partners of ferns and lycophytes (Kottke *et al.*, 2008).

Compared to the overwhelming diversity of green plants in the tropics, the studies on tropical mycorrhizae are relatively few (Wang and Qiu, 2006). One area worthy of such investigations is the Reserva Biológica San Francisco in southern Ecuador (Prov. Zamora-Chinchipe), where we conducted ecological studies on ferns and lycophytes (Gradstein *et al.*, 2007). The 1000 ha large reserve contains mature montane rain forest at 1800–3150 m and harbors 247 species of ferns (incl. horsetails; Smith *et al.*, 2006) and lycophytes (Lehnert *et al.*, 2007). The rugged topography of the area creates a mosaic of different substrate properties, with nutrient deficient soils on the ridges (Gradstein *et al.*, 2008) and slopes that receive a downhill flow of nutrients (Wilcke *et al.*, 2001). The divergent soil properties should also influence the mycorrhization of the plant species, given the fact that mycorrhizae enable plants to prosper in harsh nutrient deficient environments (Cairney and Meharg, 2003). Surprisingly, many usually epiphytic species in the area also colonize the ground on the ridges (Kessler and Lehnert, 2009), although epiphytic ferns are considered to be less dependent on mycorrhizae than terrestrial ones. Highly abundant groups with numerous epiphytic species in the area are the filmy ferns (Hymenophyllaceae), grammitid ferns (Polypodiaceae), and the genus *Elaphoglossum* (Dryopteridaceae).

Looking for a reference on the mycorrhizal status for these fern groups, we found that most available reports are for smaller regions outside of South America (e.g., Berch and Kendrick 1982; Cooper 1976; Gemma *et al.*, 1992; Iqbal *et al.*, 1981; Moteetee *et al.*, 1996; Nadarajah and Nawawi, 1993), and the few surveys cover only a fraction of the ferns and lycophytes worldwide (Boullard, 1958, 1979; Hepden, 1960; Newman and Reddell, 1987). No treatment for tropical Andean ferns was found; the few studies in South and Central America had either no overlap in the investigated species (Andrade *et al.*, 2000; Fernández 2005), or they had contradicting results for the same species (Lesica and Antibus, 1990; Schmid *et al.*, 1995). Compared to the general diversity, the number of investigated species from our three focal groups (filmy ferns, grammitid ferns, and the genus *Elaphoglossum*) is rather low. The present account aims to increase the investigated species number of these groups in order to have a more representative basis for future comparative studies.

Boullard (1958) included several Neotropical species in his survey but these were sampled either from herbarium specimens or from cultivated material. Drying reduces the ability of the hyphae to take up the dye, so that the mycorrhization of the plant may be rated too low or may go undetected. In cultivation, the kind or degree of mycorrhization may depend on the fertilization of the substrates (Entry *et al.*, 2002). Species that otherwise are mycorrhizal may completely dispense of the symbiosis in cultivation. Therefore, root samples are best taken directly from nature and preserved specifically for later dyeing. As far as we know, this is the first survey on mycorrhizae in tropical Andean ferns sampled *in situ*.

Materials and Methods

Root samples were collected at different sites in SE Ecuador: A) along the Gualaceo-Limon road (3100–3300 m, Prov. Azuay), B) the mountain pass El Tiro between the towns of Loja and Zamora (2600–2800 m, Prov. Loja/Zamora-Chinchipe), C) the area of Cerro Toledo, situated E of the town of Yantzatza (2900–3100 m, Prov. Loja), D) Reserva Biológica San Francisco (1800–2600 m, Prov. Zamora-Chinchipe), E) Reserva Cajanuma (2750 m, Prov. Loja), F) Reserva Tapichalaca (2450–2650 m, Prov. Zamora-Chinchipe), and G) the Campamento Indígena Shaimi on the shores of Río Nangaritza (900–1200 m, Prov. Zamora-Chinchipe). The study sites span an elevational gradient of 2400 m and range from lower montane forest to páramo vegetation. All sample areas face east and receive heavy precipitation all year round (Richter, 2003).

Sampling was focused on previously rarely investigated taxa. The substrates of the ferns were categorized as terrestrial, epiphytic, and saxicolous (= epilithic, rupicolous). Voucher specimens were deposited at Pontificia Universidad Católica del Ecuador, Quito (QCA). Duplicate collections of M. Lehnert were further distributed to Göttingen (GOET) and Berkeley (UC), and a set of specimens collected by L. Pazmiño is deposited at the herbarium of Universidad Técnica Particular de Loja (UTPL), Ecuador.

Sample plants were carefully removed and cleaned mechanically from the substrate, then rinsed with water to remove smaller litter parts and mineral compounds. At least 10 cm of roots from each specimen were preserved in 70% ethanol; of plants which we suspected to harbor DSE, additional 5–10 cm of the roots were preserved in 10% aqueous glutardialdehyde for transmission electron microscopy (TEM) preparation and stored at 8–10°C.

Preparation of the ethanolic samples for light microscopy followed Grace and Stribley (1991) and Haug *et al.* (2004). The samples were cleared in 10% KOH for ca. 24 h at 60°C; if the roots were still dark, the KOH was changed and the sample was kept at 60°C for another 12–24 h. Then the roots were rinsed twice with water and acidified with 1 N HCl. Staining was done with 0.05% methyl blue in lactic acid for at least 3 h. The stained roots were examined with a dissecting microscope at 30–60 ×; promising young roots were cut into portions, mounted on slides in lactic acid and examined at 100–400 ×. If mounted roots turned out to be insufficiently cleared, they were bleached with 3 % H_2O_2 for 2–5 min, rinsed with water and acidified with 1 N HCl. Then they were covered with same staining solution as before and heated over a small flame for 1–3 min. Excess staining solution was washed off with 90% lactic acid.

Preparation of the TEM samples followed Schmid *et al.* (1995). We opted for the fixation with 1% osmiumtetroxid for 1 h at 20°C, then 1% uranylacetate for 1 h at 20°C. Samples and slides are stored at the Georg-August-Universität Göttingen, Germany.

AMF were screened in the light microscope for presence. AMF are recognizable as relatively strong, aseptate hyphae with irregular diameter, forming terminal and lateral vesicles (Boullard, 1958). These infections were counted as real mycorrhizae if arbuscules were visible in the cortex (Gemma *et al.*, 2002).

Dark septate endopyhtes (DSE) were assigned to ascomycetes (Schmid *et al.*, 1995) if the characteristic Woronin bodies at the porate septa in the hyphae were visible in the TEM (Fig. 1D; Haug *et al.*, 2004). Fungal infection was considered as mycorrhiza if hyphal coils were developed in host cells that were still intact and showed some response to the infection, i.e., thickening of the cell walls where the hyphae penetrated the cell and thickening of host cell cytoplasma as indicator of increased cytological activity (Fig. 1C).

The frequency of infections in the roots was quantified under the light microscope, preferably on a single root with a minimum length of 10 cm measured from the root tips. In cases where the plants developed only considerably shorter roots, we combined several complete roots to reach the minimum length of 10 cm. The frequency of stained hyphae was categorized in three classes (Gemma *et al.*, 1992) to give an impression of the extent of the infection: Present in 1) <25%, 2) 25–75%, and 3) >75% of investigated root length. Presence of single hyphae or vesicles in the outer cortex as well as infection rates below 5% were considered as erroneous infections and not counted as mycorrhizal association. We did not distinguish between "obligately" and "facultatively mycorrhizal" because we usually sampled

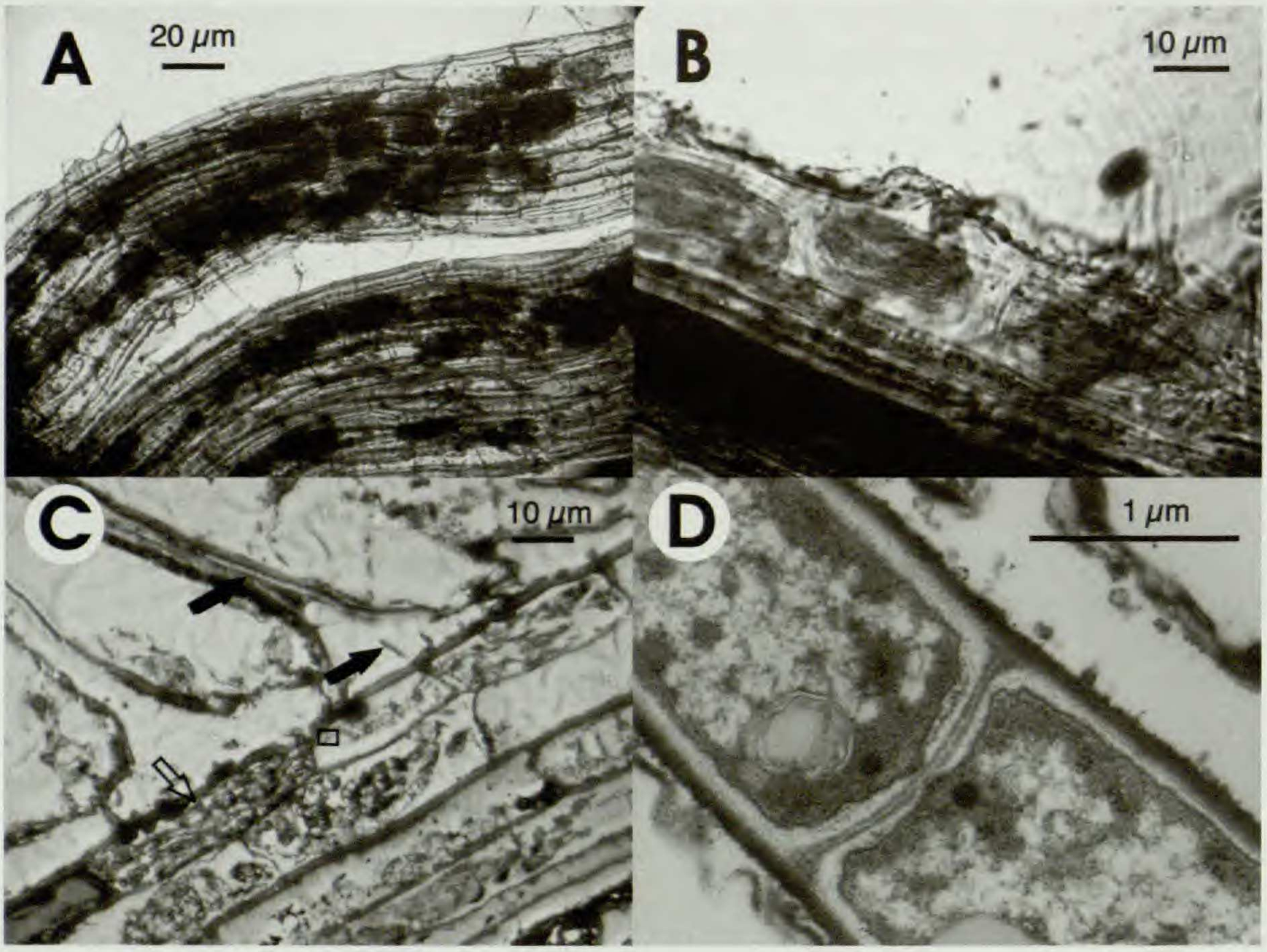

FIG. 1. Fungal infections in fern roots. A) AMF infection in young roots of *Loxsomopsis pearcei*; arbuscules fill out most of the cortical cells. Photograph M. Lehnert; B) DSE infection of root cortex in *Lellingeria major*, visible as external net and dense, internal coils of hyphae. Photograph M. Lehnert; C) Infection by ascomycete in *Melpomene firma*; the hyphae enter the root through the root hair tips (black arrows) and form dense hyphal coils in the outer cortex (outlined arrow). Photograph M. Lehnert; D) Infection by ascomycete in *Melpomene firma*; detail of septum with characteristic Woronin-bodies, visible as darker dot on each side of the septum. TEM-photograph I. Kottke.

only one specimen per species and habitat. Since degree and frequency of mycorrhization is dependent on external factors, such a categorization would be misguiding.

RESULTS

Among the 101 Ecuadorian fern samples, 85 species from 10 families were represented (Table 1). A total of 63 samples were infected by mycorrhizal fungi. AMF occurred in 19 species (22.35%) represented by 19 samples, and 36 species (42.35%) represented by 44 samples were infected by dark DSE (Table 2). Identified DSE always turned out to be ascomycetes that probably form a mycorrhizal association similar to the ericoid mycorrhiza (Schmid *et al.*, 1995; Kottke, 2002). Since it was not possible to process all specimens in question adequately, we retain the more general term DSE in the following

passages. The roots of 35 samples were free of evident fungal infection. Three specimens (*Arachniodes denticulata*, *Elaphoglossum lloense*, *Micropolypodium sp.*) had only a weak peripheral infection by DSE. They were regarded as dubious and are included in the non-mycorrhizal species (35.30% of the species). Mixed infections cannot be confidently reported.

AMF were found in 29.10% and 28.57% of the terrestrial and saxicolous species, respectively, but only in 3.45% of the epiphytes (Table 2). DSE showed a similar presence in terrestrial and saxicolous species (30.91% and 28.57%, respectively), but they dominated over AMF in the epiphytic species with 58.62%.

Hymenophyllaceae were represented with 18 species in our sample and showed a high presence of mycorrhization (78%). The mainly epiphytic species of *Hymenophyllum* were colonized by DSE (80%), whereas the predominantly terrestrial or saxicolous species of *Trichomanes* s.l. (*Trichomanes*, *Abrodyctium*) had more cases of AMF infection (50%). One unidentified *Trichomanes* grew epiphytically and had DSE like the epiphytic *Hymenophyllum* species. The only terrestrial *Trichomanes* s.l. with DSE was *Trichomanes dactylites* Sodiro.

Grammitid ferns (Polypodiaceae; Schneider *et al.*, 2004, Smith *et al.*, 2006), represented by 24 species, had an infection rate of 75%. Only ascomycetes (i.e., DSE) were found as fungal partner, even in terrestrial and saxicolous species (L. Pazmiño, unpubl. data). Non-grammitid Polypodiaceae were completely free of evident fungal infections.

Among the 23 species of *Elaphoglossum*, we found only 12 (52.20%) with fungal infection. DSE accounted for 75% of the infections.

The remainder of the investigated species showed mycorrhizal associations as was more or less expected from previous accounts. All three species of *Asplenium* (Aspleniaceae) were terrestrial and free of fungal infection. Of the two terrestrial species of *Blechnum* (Blechnaceae), only one had a low AMF infection. The investigated Pteridaceae showed a medium to strong infection by AMF (2 species, 100% infection).

Although they have been cited as examples for high infection rates (Boullard, 1958, 1979), only 50% of the species in the Cyatheaceae and 40% of the species in the Gleicheniaceae had mycorrhizal associations (Table 1). However, the exclusive colonization by AMF could be confirmed in both families.

Our sample size was not sufficient for a statistical analysis of changing mycorrhization along an elevational gradient. The localities of the samples are included in Table 1 for future studies focusing on this topic, which may want to include the data presented here.

Discussion

The overall infection by confirmed and putatively mycorrhizal fungi among our samples was 62.38% (64.70% at the species level). These percentages are lower than those reported for angiosperms or land plants in general. Trappe

TABLE 1. Investigated samples. Abbreviations: t = terrestrial, e = epiphyte, s = saxicolous; AMF = arbuscular mycorrhizal fungi; DSE = dark septate endophytes; ? = dubious record. Localities: A) Gualaceo-Limon road (3100–3300 m), B) mountain pass El Tiro (2600–2800 m), C) Cerro Toledo (2900–3100 m, Prov. Loja), D) Reserva Biológica San Francisco (1800–2600 m), E) Reserva Cajanuma (2750 m), F) Reserva Tapichalaca (2450–2650 m), G) the Campamento Indígena Shaimi (900–1200 m).

Species	Sub-strate	Fungal infection (%)	Type of infection	Collection	Loc.
Aspleniaceae					
Asplenium auritum Sw.	t	-	-	Lehnert M. 1473	F
Asplenium hallii Hook.	t	-	-	Lehnert M. 1395	B
Asplenium serra Langsd. & Fisch.	t	-	-	Lehnert M. 1394	B
Blechnaceae					
Blechnum schomburgkii (Klotzsch) C. Chr.	t	5–25	AMF	Lehnert M. 1484	D
Blechnum sp. 1	t	-	-	Lehnert M. 1440	D
Cyatheaceae					
Alsophila conantiana Lehnert	t	5–25	AMF	Lehnert M. 1414	B
Cyathea bipinnatifida (Baker) Domin	t	-	-	Lehnert M. 1438	D
Cyathea dudleyi R. M. Tryon	t	5–25	AMF	Lehnert M. 1550	D
Cyathea hybrid	t	-	-	Lehnert M. 1434	D
Cyathea obnoxia Lehnert	t	-	-	Lehnert M. 1470	F
Cyathea peladensis (Hieron.) Domin	t	5–25	AMF	Lehnert M. 855	D
Dryopteridaceae					
Arachniodes denticulata (Sw.) Ching	t	<5	DSE?	Lehnert M. 996	F
Elaphoglossum antisanae (Sodiro) H. Christ	e	-	-	Lehnert M. 1485	D
Elaphoglossum argyrophyllum (Sodiro) R. C. Moran, comb. ined.	e	-	-	Lehnert M. 1490	D
Elaphoglossum deltoideum (Sodiro) H. Christ	t	-	-	Lehnert M. 1460	C
Elaphoglossum deltoideum (Sodiro) H. Christ	t	-	-	Lehnert M. 1463	C
Elaphoglossum dendricola (Baker) H. Christ	t	-	-	Lehnert M. 1458	C
Elaphoglossum engelii (H. Karst.) H. Christ	t	25–75	DSE	Lehnert M. 1457	C
Elaphoglossum erinaceum (Fée) T. Moore	e	5–25	DSE	Lehnert M. 1387	B
Elaphoglossum glossophyllum Hieron.	e	-	-	Lehnert M. 1551	D
Elaphoglossum glossophyllum Hieron.	e	5–25	DSE	Lehnert M. 1552	D
Elaphoglossum guamanianum (Sodiro) C. Chr.	e	25–75	DSE	Lehnert M. 1493	D
Elaphoglossum heteromorphum (Klotzsch) T. Moore	t	5–25	AMF	Lehnert M. 1462	C

TABLE 1. Continued.

Species	Sub-strate	Fungal infection (%)	Type of infection	Collection	Loc.
Elaphoglossum latifolium (Sw.) J. Sm.	e	25–75	AMF	Lehnert M. 1492	D
Elaphoglossum lloense (Hook.) T. Moore	e	<5	DSE?	Lehnert M. 1491	D
Elaphoglossum papillosum (Baker) H. Christ	t	5–25	AMF	Lehnert M. 1472	F
Elaphoglossum petiolosum (Desv.) T. Moore	e	5–25	DSE	Lehnert M. & N. Mandl 1441	B
Elaphoglossum productum Rosenst.	e	-	-	Lehnert M. 1553	D
Elaphoglossum quitense (Baker) C. Chr.	t	25–75	DSE	Lehnert M. 1459	C
Elaphoglossum sp. 1	e	5–25	DSE	Lehnert M. 1487	D
Elaphoglossum sp. 2	e	-	-	Lehnert M. 1486	D
Elaphoglossum sp. 3	t	-	-	Lehnert M. 1502	D
Elaphoglossum squarrosum (Klotzsch) T. Moore	t	5–25	AMF	Lehnert M. 1488	D
Elaphoglossum vulcanicum H. Christ	e	-	-	Lehnert M. 1475	F
Elaphoglossum yatesii (Sodiro) H. Christ	t	25–75	DSE	Lehnert M. 1461	C
Lastreopsis kilippii (Maxon) Tindale	t	25–75	AMF	Lehnert M. 980	F
Polystichum platyphyllum (Willd.) C. Presl	t	25–75	AMF	Lehnert M. 1412	B
Gleicheniaceae					
Sticherus brevitomentosus B. Øllg. & Østergaard	t	-	-	Lehnert M. 1480	F
Sticherus melanoblastus Østergaard & B. Øllg.	t	-	-	Lehnert M. 1549	D
Sticherus melanoblastus Østergaard & B. Øllg.	t	-	-	Lehnert M. 1476	F
Sticherus rubignosus (Mett.) Nakai	t	-	-	Lehnert M. 1268	F
Sticherus rubignosus (Mett.) Nakai	t	5–25	AMF	Lehnert M. 1478	F
Sticherus sp. 1	t	5–25	AMF	Lehnert M. 1479	F
Sticherus tomentosus (Cav. ex Sw.) A. R. Sm.	t	-	-	Lehnert M. 1477	F
Hymenophyllaceae					
Abrodictyum rigidum (Sw.) Ebihara & Dubuisson	t	25–75	AMF	Lehnert M. 1515	G
Hymenophyllum calodictyon Bosch	e	5–25	DSE	Lehnert M. 1443	B
Hymenophyllum cristatum Hook. & Grev.	e	5–25	DSE	Lehnert M. 1547	D
Hymenophyllum fucoides (Sw.) Sw.	e	5–25	DSE	Lehnert M. 1444	B
Hymenophyllum microcarpum Desv.	t	5–25	DSE	Lehnert M. 1494	D
Hymenophyllum multialatum C. V. Morton	e	25–75	DSE	Lehnert M. 1447	B
Hymenophyllum plumierii Hook. & Grev.	e	25–75	DSE	Lehnert M. 1362	B

TABLE 1. Continued.

Species	Sub-strate	Fungal infection (%)	Type of infection	Collection	Loc.
Hymenophyllum polyanthos (Sw.) Sw.	e	5–25	DSE	Lehnert M. 1445	B
Hymenophyllum sp. 1	e	-	-	Lehnert M. 1455	C
Hymenophyllum sp. 2	s	-	-	Lehnert M. 1566	A
Hymenophyllum trichomanoides Bosch	e	5–25	DSE	Lehnert M. 1446	B
Trichomanes cellulosum Klotzsch	t	5–25	AMF	Lehnert M. 1481	D
Trichomanes dactylites Sodiro	t	5–25	DSE	Lehnert M. 1501	D
Trichomanes elegans Rich.	s	5–25	AMF	Lehnert M. 1516	G
Trichomanes pellucens Kunze	t	5–25	AMF	Lehnert M. 1514	G
Trichomanes sp. 1	e	5–25	DSE	Lehnert M. 1483	D
Trichomanes sp. 2	s	-	-	Lehnert M. 1546a	G
Trichomanes sp. 3	t	-	-	Lehnert M. 1482	D
Loxomataceae					
Loxsomopsis pearcei (Maxon) Baker	t	>75	AMF	Lehnert M. 1056	F
Polypodiaceae [non-grammitids]					
Campyloneurum amphostenon Fée	t	-	-	Pazmiño L. s.n.	D
Niphidium albopunctatissimum Lellinger	t	-	-	Pazmiño L. s.n.	D
Pleopeltis percussa Hook. & Grev.	t	-	-	Pazmiño L. s.n.	D
Polypodiaceae [grammitids]					
Ceradenia farinosa (Forssk.) Kaulf.	e	25–75	DSE	Pazmiño L. s.n.	D
Ceradenia farinosa (Forssk.) Kaulf.	t	5–25	DSE	Pazmiño L. s.n.	D
Ceradenia glabra A. R. Smith & M. Kessler	e	5–25	DSE	Lehnert M. 1495	D
Cochlidium serrulatum (Sw.) L. E. Bishop	t	5–25	DSE	Lehnert M. 1467	C
Cochlidium serrulatum (Sw.) L. E. Bishop	e	5–25	DSE	Pazmiño L. s.n.	D
Enterosora parietina (Klotzsch) L.E. Bishop	e	-	-	Lehnert M. 1497	D
Grammitis paramicola L. E. Bishop	e	25–75	DSE	Pazmiño L. s.n.	D
Grammitis paramicola L. E. Bishop	t	25–75	DSE	Pazmiño L. s.n.	D
Lellingeria major (Copel.) A. R. Sm. & R.C. Moran	t	25–75	DSE	Lehnert M. 1466	C
Lellingeria major (Copel.) A. R. Sm. & R.C. Moran	e	25–75	DSE	Lehnert M. 1498	D
Lellingeria subsessilis (Baker) A. R. Sm. & R. C. Moran	e	-	-	Lehnert M. 1499	D
Lellingeria subsessilis (Baker) A. R. Sm. & R. C. Moran	e	25–75	DSE	Pazmiño L. s.n.	D
Lellingeria subsessilis (Baker) A. R. Sm. & R. C. Moran	t	5–25	DSE	Pazmiño L. s.n.	D

TABLE 1. Continued.

Species	Sub-strate	Fungal infection (%)	Type of infection	Collection	Loc.
Melpomene assurgens (Maxon) A. R. Sm. & R. C. Moran	e	25–75	DSE	Lehnert M. 1427	B
Melpomene erecta (C. V. Morton) A. R. Sm. & R. C. Moran	t	5–25	DSE	Lehnert M. 1570	A
Melpomene firma (J. Sm.) A. R. Sm. & R. C. Moran	e	25–75	DSE	Lehnert M. 1328	B
Melpomene gracilis (Hook.) A. R. Sm. & R. C. Moran	s	5–25	DSE	Lehnert M. 1569	A
Melpomene moniliformis (Lagasca ex Sw.) A.R. Sm. & R.C. Moran	e	5–25	DSE	Lehnert M. 1559	A
Melpomene moniliformis (Lagasca ex Sw.) A.R. Sm. & R.C. Moran	t	5–25	DSE	Lehnert M. 1510	E
Melpomene occidentalis Lehnert	t	-	-	Lehnert M. 1507	E
Melpomene occidentalis Lehnert	t	-	-	Lehnert M. 1508	E
Melpomene pseudonutans (Christ & Rosenst.) A.R. Sm. & R.C. Moran	s	5–25	DSE	Lehnert M. 1558	A
Melpomene pseudonutans (Christ & Rosenst.) A.R. Sm. & R.C. Moran	t	5–25	DSE	Lehnert M. 1464	C
Melpomene pseudonutans (Christ & Rosenst.) A.R. Sm. & R.C. Moran	e	25–75	DSE	Pazmiño L. s.n.	D
Melpomene sklenarii Lehnert	t	5–25	DSE	Lehnert M. 1465	C
Melpomene wolfii (Hieron.) A. R. Sm. & R. C. Moran	e	25–75	DSE	Pazmiño L. s.n.	D
Melpomene wolfii (Hieron.) A. R. Sm. & R. C. Moran	t	5–25	DSE	Pazmiño L. s.n.	D
Micropolypodium sp. 1	t	<5	DSE?	Pazmiño L. s.n.	D
Micropolypodium sp. 1	e	5–25	DSE	Pazmiño L. s.n.	D
Terpsichore lanigera (Desv.) A. R. Sm.	e	-	-	Lehnert M. 1496	D
Terpsichore leucosticta (J. Sm.) A. R. Sm.	t	5–25	DSE	Lehnert M. 1509	E
Terpsichore semihirsuta (Klotzsch) A. R. Sm.	t	5–25	DSE	Lehnert M. 1511	E
Pteridaceae					
Pteris muricata Hook.	t	5–25	AMF	Lehnert M. 1571	B
Pterozonium brevifrons (A. C. Sm.) Lellinger	s	25–75	AMF	Lehnert M. 1435	D
Thelypteridaceae					
Thelypteris minutula C. V. Morton	s	-	-	Lehnert M. 1337	B

TABLE 2. Distribution of the 85 investigated species onto the registered categories: total numbers are followed by percentages per life forms (columns) and infection types (rows) in brackets. Abbreviations are the same as in Table 1; NM = non-mycorrhizal; ** six species occurred on more than one substrate, adding 7% to the total count and 20% to NM.

Species	All	t	e	s
total	85 (100/100**)	55 (100/64.71**)	29 (100/34.12**)	7 (100/8.24**)
AMF	19 (22.35/100)	16 (29.10/84.21)	1 (3.45/5.26)	2 (28.57/10.53)
DSE	36 (42.35/100)	17 (30.91/47.22)	17 (58.62/47.22)	2 (28.57/5.55)
NM	30 (35.30/100**)	22 (35.29/73.33**)	11 (37.93/36.67**)	3 (42.86/10.00**)

(1987) estimated that 82% of angiosperms host mycorrhizae; Wang and Qiu (2006) concluded that 80% of all land plants are mycorrhizal.

Studies focusing on ferns and lycophytes found similar results to ours. Values gathered from literature (Boullard, 1958; Cooper, 1976; Berch and Kendrick, 1982, Iqbal *et al.*, 1981, Gemma *et al.*, 1992, Lesica and Antibus, 1990, Moteetee *et al.*, 1996; Ragupathy and Mahadevan, 1993, Schmid *et al.*, 1995, Muthukumar and Udaiyan, 2000; Zhao, 2000; Zhang *et al.*, 2003) sum up to 68% of general fungal colonization and to 53% of AMF in ferns and lycophytes (M. Lehnert, unpubl. data). Wang and Qiu (2006), considering only AMF, found a comparable 52% of the species of ferns and lycophytes to be mycorrhizal.

Despite the congruence in general mycorrhizal infection, our survey found AMF in only 22.35% of the species, including 29.10% of the terrestrial, 28.57% of the saxicolous species, and only 3.45% of the epiphytes. In contrast, DSE showed a similar presence in terrestrial and saxicolous species (30.91% and 28.57%), but they dominated over AMF in the epiphytic species with 58.62%.

The discrepancy in AMF percentages between our study and the average is likely due to our selective sampling. We laid the focus on predominantly epiphytic taxa, and although we still examined more terrestrial than epiphytic samples, we evidently included a higher percentage than previous studies. The epiphytic habitat is rarely colonized by AMF because their spores are not easily dispersed from the soil. Furthermore, most AMF are dependent on their host, requiring the presence of a facultatively mycorrhizal plant for successfully establishing the symbiosis on a chorophyte (Janos, 1993). Thus the low presence of AMF in epiphytes (3.45%) is not surprising. DSE, however, have spores that get airborne and are thus more likely to contact the roots of epiphytic plants. Epiphytic plant species are well known to suffer from nutrient shortages and should greatly benefit from a fungal symbiont (Lesica and Antibus, 1990). If DSE are excluded in surveys as potential mycorrhizal partners in ferns and lycophytes (Lesica and Antibus, 1990; Michelsen, 1993), the recorded mycorrhization is low or absent, in our case only 22.4%. If they are regarded as mycorrhizae, the mycorrhization level will increase (Schmidt *et al.*, 1995; Kottke, 2002), in our case to 42.35%. Overall, the degree of

infection by DSE was higher in our study than in any other previous study on ferns and lycophytes.

Beyond these general patterns, it is worthwhile to focus on individual study groups. The Hymenophyllaceae nicely mirror the general distribution pattern of the fungal infections. Terrestrial and saxicolous species have predominantly AMF, whereas DSE prevail in epiphytes. Gammitid ferns (Polypodiaceae), however, have almost exclusively DSE, no matter if they grew as epiphytes or as terrestrials. This apparent conflict with the general trend is due to the microhabitats inhabited by the species. The investigated terrestrial grammitid ferns usually grew in thick moss cushions like their epiphytic kin and by this means under very similar ecological conditions, which may lead to maintaining the type of mycorrhiza. Furthermore, most of the species sampled as terrestrials are either potentially epiphytic or closely related to epiphytic species. Only the samples of *Melpomene occidentalis* Lehnert rooted directly in mineral soil and showed no fungal infection. Opposed to this, the samples of eleven terrestrial and epiphytic species of non-grammitid Polypodiaceae from the investigated area are free of fungal infections, which is congruent with previous reports (Lesica and Antibus, 1990; Schmid *et al.*, 1995). Since grammitid ferns represent a clade nested deeply within the Polypodiaceae, it is likely that the original condition in the family is a lack of mycorrhization and that mycorrhization has been secondarily regained in grammitid ferns. Apparently, this symbiosis was developed with DSE rather than with glomeromycetes. A similar situation of loss of AMF mycorrhization and secondary gain of DSE mycorrhization, also related with shifts between the terrestrial and epiphytic habitat, has been reported in liverworts (Kottke and Nebel, 2005).

The genus *Elaphoglossum* showed no clear correlation between the types of substrate and fungal infections. The genus *Asplenium* is not very diverse or abundant in the study sites and occurred only on the lower slopes where nutrients are accumulated (Gradstein *et al.*, 2008). The absence of mycorrhizae in our samples may be related to the improved availability of nutrients at their microhabitats. Previous studies found generally low infection rates in the Aspleniaceae (e.g., Boullard, 1958) and often varying results within a species, indicating that most species may be only facultatively mycorrhizal.

Gleicheniaceae are usually axiomatic for strong presence of mycorrhizae (100%; Boullard, 1958, 1979). It is assumed that this affects both their ability to grow on nutrient deficient soils and their inability to be transplanted and cultivated. Surprisingly, we found only 40% of our samples infected by AMF. Their root samples, however, were difficult to prepare because of a tough texture and dark, persistent cortical colorants. The necessary clearing with hydrogen peroxide may have affected the colourability of fungal hyphae with dye. Possibly a higher percentage of fungal infections was present but not detectable in our samples of Gleicheniaceae.

Our results for the Cyatheaceae are much lower (50% of specimens infected) than the results of previous surveys (100% of specimens infected; Boullard, 1958; Hepden, 1960). The tree ferns (families Cyatheaceae and Dicksoniaceae)

bear the difficulty of acquiring fine roots from the compact subterranean root system that many species develop. Aerial roots from the trunks are easier to harvest but are expected to lack mycorrhizae because they are less likely to get in contact with inoculum of soil fungi. In order to bypass this sampling artefact, the plants included in this study were either small species or young plants of easily assignable larger species, which can be uprooted with most of their roots. One explanation for the low infection rate could be that these juvenile plants of *Cyathea* are less dependent on mycorrhizae than mature plants. The trunk-less tree ferns dwell in the shade where these often sun-loving species are under lesser drought stress but presumably achieve only a part of their potential photosynthetic rate. The profits of better supply with water and micronutrients may not compensate the cost of sharing assimilates with symbiotic fungi.

We are aware that negative results in any species here included do not exclude the potential occurrence of mycorrhiza in other individuals of the same species. We aim to widen our sample size and want to include conspecific samples from sites with different substrate chemistry. This should allow us not only to distinguish between facultative and obligatory mycorrhizae but also about the conditioning factors.

Acknowledgments

We thank our colleagues of the Research Unit of the DFG 402 "Functionality in a Tropical Mountain Rainforest: Diversity, Dynamic Processes and Utilization Potentials under Ecosystem Perspectives" for various help and fruitful discussion, especially Nicki Mandl and Rob Gradstein; we are indebted to our Ecuadorian counterparts in Loja (Fundación Cultura y Naturaleza; Herbario LOJA/Universidad Nacional de Loja; Universidad Técnica Particular de Loja [UTPL]) and Quito (Pontificia Universidad Católica del Ecuador [PUCE]). The Ministerio del Ambiente, Ecuador, kindly issued permits for field research and collection of plant material. Special thanks go to Robbin C. Moran, New York Botanical Garden, for determinations of the *Elaphoglossum* samples. This study was financially supported by the German Research Foundation (DFG, grant GR 1588/7).

Literature Cited

Allen, M. F., W. Swenson, J. I. Querejeta, L. M. Egerton-Warburton and K. K. Treseder. 2003. Ecology of mycorrhizae: A conceptual framework for complex interactions among plants and fungi. Annu. Rev. Phytopathol. 47:271–303.

Andrade, A. C. S., M. H. Queiroz, R. A. L. Hermes and V. L. Oliveira. 2000. Mycorrhizal status of some plants of the *Araucaria* forest and the Atlantic rainforest in Santa Catarina, Brazil. Mycorrhiza 10:131–136.

Berch, S. H. and B. Kendrick. 1982. Vesicular-arbuscular mycorrhizae of southern Ontario ferns and fern allies. Mycologia 74:769–776.

Blackwell, M. 2000. Terrestrial life - Fungal from the start? Science 289:1884–1885.

Boullard, B. 1958. La mycotrophie chez les ptéridophytes. Sa fréquence, ses caractéres, sa signification. Doctor thesis, Université de Caen. (Imprimerie E. Droulliard, Bordeaux)

Boullard, B. 1979. Consideration sur la symbiose fongique chez les Pteridophytes. Syllogeous 19:1–59.

Brundrett, K. 2002. Coevolution of roots and mycorrhizas of land plants. New Phytol. 154:275–304.

Brundrett, M. C. 2004. Diversity and classification of mycorrhizal associations. Bot. Rev. 79:473–495.

CAIRNEY, J. W. G. and A. A. MEHARG. 2003. Ericoid mycorrhiza: a partnership that exploits harsh edaphic conditions. Eur. J. Soil Sci. 54:735–740.

COOKE, J. C. and M. W. LEFOR. 1998. The mycorrhizal status of selected plant species from Conneticut wetlands and transition zones. Restoration Ecology 6:214–222.

COOPER, K. M. 1976. A field survey of mycorrhizas in New Zealand ferns. N.Z. J. Bot. 14:169–181.

ENTRY, J. A., P. T. RYGIEWICZ, L. S. WATRUD and P. K. DONNELLY. 2002. Influence of adverse soil conditions on the formation and function of arbuscular mycorrhizas. Adv. Environ. Res. 7:123–138.

FERNÁNDEZ, N., S. FONTENLA and M. I. MESSUTI. 2005. Micorrizas en pteridofitas de los bosques templado-lluviosos del Noroeste de Patagonia. *II Convención Ambiental Universitaria Patagónica.*

GEMMA, J. N., R. E. KOSKE and T. FLYNN. 1992. Mycorrhizae in Hawaiian pteridophytes: occurrence and evolutionary significance. Amer. J. Bot. 79:843–852.

GRACE, C. and D. P. STRIBLEY. 1991. A safer procedure for routine staining of vesicular-arbuscular mycorrhizal fungi. Mycol. Res. 95:1160–1162.

GRADSTEIN, S. R., M. KESSLER, M. LEHNERT, M. ABIY, N. MANDL, F. MAKESCHIN and M. RICHTER. 2008. Vegetation, climate and soil of the unique *Purdiaea* forest of southern Ecuador. Ecotropica 14:15–26.

HAUG, I., M. WEIß, J. HOMEIER, F. OBERWINKLER and I. KOTTKE. 2004. Russulaceae and Thelephoraceae form ectomycorrhizas with members of the Nyctaginaceae (Caryophyllales) in the tropical mountain rain forest of southern Ecuador. New Phytol. 165:923–936.

HEPDEN, P. M. 1960. Studies in vesicular-arbuscular endophytes. II. Endophytes in the Pteridophyta, with special reference to leptosporangiate ferns. Trans. Br. Mycol. Soc. 43:559–570.

IQBAL, S. H., M. YOUSAF and M. YOUNUS. 1981. A field survey of mycorrhizal associations in ferns of Pakistan. New Phytol 87:69–89.

JANOS, D. P. 1993. Vesicular-arbuscular mycorrhizae of epiphytes. Mycorrhiza 4:1–4.

JUMPPONEN, A. and J. M. TRAPPE. 1998. Dark septate endophytes: a review of facultative biotrophic root-colonizing fungi. New Phytol. 140:295–310.

KESSLER, M. and M. LEHNERT. 2009. Do ridge habitats contribute to pteridophyte diversity in tropical montane forests? A case study from southeastern Ecuador. J. Pl. Res. 122:421–428.

KOTTKE, I. 2002. Mycorrhizae - Rhizosphere determinants of plant communities. Pp. 919–932, in: WAISEL, Y., ESHEL, A. and U. KAFKAFI, (eds.), Plant Roots: The Hidden Half. 3rd ed. Marcel Dekker, Inc.

KOTTKE, I. and M. NEBEL. 2005. The evolution of mycorrhiza-like associations in liverworts: An update. New Phytol. 167:330–334.

KOTTKE, I., A. BECK, I. HAUG, S. SETARO, V. JESKE, J. P. SUÁREZ, L. PAZMIÑO, M. PREUßING, M. NEBEL and F. OBERWINKLER. 2008. Mycorrhizal state and new and special features of mycorrhizae of trees, ericads, orchids, ferns and liverworts. Pp. 137–148, in: BECK, E., J. BENDIX, I. KOTTKE, F. MAKESCHIN and R. MOSANDL, (eds.), Gradients in a Tropical Mountain Ecosystem of Ecuador. Series Ecological Studies 198, Springer Verlag, Berlin, Heidelberg.

LEHNERT, M., M. KESSLER, L. I. SALAZAR, H. NAVARRETE, F. A. WERNER and S. R. GRADSTEIN. 2007. Pteridophyta. Pp. 59–68, in: LIEDE-SCHUMANN, S. and S.-W. BRECKLE, (eds.), Provisional Checklists of fauna and flora of the San Francisco valley and its surroundings (Reserva San Francisco/Prov. Zamora–Chinchipe, southern Ecuador). Ecotrop. Monogr. 4.

LESICA, P. and R. K. ANTIBUS. 1990. The occurrence of mycorrhizae in vascular epiphytes of two Costa Rican rain forests. Biotropica 33:250–258.

MICHELSEN, A. 1993. The mycorrhizal status of vascular epiphytes in Bale Mountains National Park, Ethiopia. Mycorrhiza 4:11–15.

MOTEETEE, A., J. G. DUCKETT and A. J. RUSSELL. 1996. Mycorrhizas in the ferns of Lesotho. Pp. 621–631, CAMUS, J. M., M. GIBBY and R. J. JOHNS, (eds.), Pteridology in perspective. Royal Botanic Gardens, Kew.

MUTHUKUMAR, T. and K. UDAIYAN. 2000. Arbuscular mycorrhizas of plants growing in the Western Ghats region, Southern India. Mycorrhiza 9:297–313.

NADARAJAH, P. and A. NAWAWI. 1993. Mycorrhizal status of epiphytes in Malaysian oil palm plantations. Mycorrhiza 4:21–24.

NEWMAN, E. I. and P. REDDELL. 1987. The distribution of mycorrhizas among families of vascular plants. New Phytol. 106:745–751.

PIROZYNSKI, K. A. and D. W. MALLOCH. 1975. The origin of land plants: A matter of mycotrophism. BioSystems 6:153–164.

RAGUPATHY, S. and A. MAHADEVAN. 1993. Distribution of vesicular-arbuscular mycorrhizae in the plants and rhizosphere soils of the tropical plains, Tamil Nadu, India. Mycorrhiza 3:123–136.

RICHTER, M. 2003. Using epiphytes and soil temperatures for eco-climatic interpretations in southern Ecuador. Erdkunde 57:161–181.

SCHMID, E., F. OBERWINKLER and L. D. GÓMEZ. 1995. Light and electron microscopy of a host-fungus interaction in the roots of some epiphytic ferns from Costa Rica. Can. J. Bot. 73:991–996.

SCHNEIDER, H., A. R. SMITH, R. CRANFILL, T. J. HILDEBRAND, C. H. HAUFLER and T. A. RANKER. 2004. Unraveling the phylogeny of polygrammoid ferns (Polypodiaceae and Grammitidaceae): exploring aspects of diversification of epiphytic plants. Mol. Phylo. Evol. 31:1041–1063.

SCHÜßLER, A., D. SCHWARZGOTT and C. WALKER. 2001. A new fungal phylum, the Glomeromycota: phylogeny and evolution. Mycol. Res. 105:1413–1421.

SMITH, A. R., K. M. PRYER, E. SCHUETTPELZ, P. KORALL, H. SCHNEIDER and P. G. WOLF. 2006. A classification for extant ferns. Taxon 55:705–731.

TRAPPE, J. M. 1987. Phylogenetic and ecologic aspects of mycotrophy in the angiosperms from an evolutionary standpoint. Pp. 5–25, in: SAFIR, G. R., (ed.), Ecophysiology of va mycorrhizal plants. Boca Raton, FL, USA.CRC Press.

WANG, B. and Y.-L. QIU. 2006. Phylogenetic distribution and evolution of mycorrhizas in land plants. Mycorrhiza 16:299–363.

WILCKE, W., S. YASIN, C. VALAREZO and W. ZECH. 2001. Nutrient budget of three microcatchments under tropical montane forest in Ecuador. Die Erde 132:61–74.

ZHANG, Y., L.-D. GUO and R.-J. LIU. 2004. Arbuscular mycorrhizal fungi associated with common pteridophytes in Dujiangyan, southwest China. Mycorrhiza 14:25–30.

ZHAO, Z. W. 2000. The arbuscular mycorrhizas of pteridophytes in Yunnan, southwest China: evolutionary interpretations. Mycorrhiza 10:145–149.